

# EVALUATION OF AUTOMATED YEAST IDENTIFICATION SYSTEM

Final Report  
NASA/ASEE Summer Faculty Fellowship Program - 1995  
Johnson Space Center

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Division: Medical Sciences  
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Date Submitted: August 10, 1995  
Contract Number: NGT-44-001-800

## ABSTRACT

One hundred and nine teleomorphic and anamorphic yeast isolates representing approximately 30 taxa were used to evaluate the accuracy of the Biolog yeast identification system. Isolates derived from nomenclatural types, environmental, and clinical isolates of known identity were tested in the Biolog system. Of the isolates tested, 81 were in the Biolog database. The system correctly identified 40, incorrectly identified 29, and was unable to identify 12. Of the 28 isolates not in the database, 18 were given names, whereas 10 were not. The Biolog yeast identification system is inadequate for the identification of yeasts originating from the environment during space program activities.

## INTRODUCTION

A ground-based automated system is needed for the identification of environmental yeasts recovered from the space shuttle, MIR, and crew members. There is a pressing need to have the capability to identify a broad spectrum of yeasts in an efficient and timely manner. The identity of environmentally important yeasts has a major impact upon accessing environmental issues and crew health.

Biolog Inc. (Hayward, CA) has recently introduced an automated system designed to identify 267 species of environmental yeasts. Using 94 biochemical tests contained in a microtiter plate, a computer and microtiter plate reader, the system can identify yeast isolates based upon their metabolic ability to utilize or oxidize different carbon sources within 24 to 72 hours. Owing to the design of the automated Biology system, its methodology may lend itself to evaluating various disinfectants for clearing the environment of yeasts and moulds.

The purpose of this investigation was to determine the accuracy of the Biolog yeast identification system for identifying clinically and environmentally important yeasts.

## MATERIALS AND METHODS

Ascomycetous and basidiomycetous yeasts of known identity (Table 1) having JSC, UTMB, ATCC, and CBS accession numbers originating from environmental and clinical sources were grown on Sabouraud glucose agar at 25°C. Subcultures were made onto Biolog Universal Yeast Agar (BUY agar, Biolog, Inc., Hayward, CA) and then incubated 48-72 hrs at 25°C. Yeast cells were harvested with a sterile cotton swab and placed into 50 ml sterile distilled water. The suspensions were adjusted to between 62 and 68.5% T at 590 nm using a spectrophotometer. The % T range was determined by using Biolog yeast turbidity standards. The suspensions were dispensed using an 8-channel repeating pipetter. 100 µl was dispensed to each well of the Biology YT MicroPlates, after which they were placed in an incubator at 25°C. Each plate was read at 24, 48, and 72 hrs using the MicroLog 3 automated MicroStation System consisting of a reading device, computer, and software release 3.50.

## RESULTS

After each reading was made, the goodness of match of the unknown isolate to the data contained within the computer database was determined. A similarity index of greater than 0.75 at 24 hrs, or greater than 0.50 at 48 and 72 hrs was considered to be an acceptable identification by the system (Table 1). Only the identifications at 72 hrs were used in this study because some isolates with subsequent periods of incubation changed

their identifications from correct to incorrect, whereas others changed from incorrect to correct.

Seventy-six isolates included in the database resulted in 40 correct identifications, 29 incorrect identifications, and 12 no identifications. For the 28 isolates not included in the database, 18 were given incorrect identifications, whereas 10 were not identified.

## DISCUSSION

Yeast identification systems must be able to accurately identify isolates included in their databases and exclude, without identifying, isolates not in their databases. Systems should provide timely identifications at a reasonable cost.

A major short coming of the Biolog system, as well as other commercial systems, is that they exclusively rely upon the ability of a yeast to assimilate or oxidize different carbon sources. Owing to the fact that morphology is a component of the description of yeast taxa, this must be considered when attempting to identify an unknown isolate. The omission of morphologic data can contribute significantly to erroneous identifications.

When considering isolates contained with the Biolog database, 40 (49.4%) were correctly identified, 29 (35.8%) were incorrectly identified, and 12 (14.8%) were not identified because the biocodes could not be matched in the Biolog database. If isolates not in the database are considered, as well as those being force-fitted into an identification, the accuracy of the Biolog system for yeast identification becomes 36.7%.

It is unfortunate that the Biolog system has no mechanism to exclude isolates whose identity and biocode are not contained within the database. This is also a problem with other systems such as Vitek and API 20C. The use of morphology would enhance the ability of these systems not only to exclude taxa, but to more accurately identify ones contained in their databases.

In contemporary mycology, the holomorphic concept is being strongly endorsed. Because many potential users of the Biolog system may not be aware of teleomorph-anamorph connections, the use of teleomorph names for many of the taxa without a cross-referenced list of teleomorph-anamorph connections creates confusion. Many of the isolates to be identified are recovered only as anamorphs. This means that most individuals will be familiar with anamorphic names rather than teleomorphic names being applied to the whole fungus.

## CONCLUSIONS

The accuracy of the Biolog System for the identification of environmental yeasts is unsatisfactory. Until the database for the system is revised, the system has only limited epidemiologic application.

Table 1.- Yeast taxa and their identification at 24, 48, and 72 hours.

Name	JSC	24 hr	48 hr	72 hr
<i>Candida albicans</i>	1	none	<i>Hyphopichia burtonii</i>	<i>Candida albicans</i>
<i>Candida krusei</i>	2	none	none	<i>Issatchenkia scutulata</i>
<i>Candida tropicalis</i>	3	<i>Candida tropicalis</i>	none	<i>Candida albicans</i>
<i>Candida kefyr</i>	4	<i>Kluyveromyces marxianus</i>	<i>Kluyveromyces marxianus</i>	<i>Kluyveromyces marxianus</i>
<i>Candida guilliermondii</i>	5	none	none	<i>Pichia guilliermondii</i>
<i>Torulopsis glabrata</i>	6	none	<i>Pichia trehalophila</i>	<i>Candida glabrata</i>
<i>Cryptococcus neoformans</i>	7	none	<i>Yarrowia lipolytica</i>	none
<i>Saccharomyces cerevisiae</i>	8	<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces cerevisiae</i>
<i>Lodderomyces elongisporus</i>	9	none	<i>Lodderomyces elongisporus</i>	none
<i>Bullera alba</i>	10	<i>Cryptococcus</i> sp.	<i>Bulleromyces albus</i>	<i>Bulleromyces albus</i>
<i>Debaryomyces maramus</i>	11	none	<i>Debaryomyces maramus</i>	<i>Debaryomyces maramus</i>
<i>Kluyveromyces marxianus</i>	12	none	<i>Kluyveromyces marxianus</i>	<i>Kluyveromyces marxianus</i>
<i>Kluyveromyces marxianus</i>	13	<i>Kluyveromyces marxianus</i>	<i>Kluyveromyces marxianus</i>	<i>Kluyveromyces marxianus</i>
<i>Kluyveromyces marxianus</i>	14	<i>Kluyveromyces marxianus</i>	<i>Kluyveromyces marxianus</i>	<i>Kluyveromyces marxianus</i>
<i>Pichia ohmeri</i>	15	none	<i>Pichia</i> sp.	<i>Pichia membranaefaciens</i>
<i>Pichia membranaefaciens</i>	16	<i>Pichia ohmeri</i>	<i>Pichia ohmeri</i>	<i>Pichia ohmeri</i>
<i>Issatchenkia orientalis</i>	17	none	<i>Issatchenkia orientalis</i>	<i>Candida sorboxylosa</i>
<i>Pichia guilliermondii</i>	18	none	<i>Kluyveromyces thermotolerans</i>	<i>Saccharomyces cerevisiae</i>

Table 1.- Yeast taxa and their identification at 24, 48, and 72 hours.

Name	JSC	24 hr	48 hr	72 hr
<i>Hansenula californica</i>	19	none	none	<i>Candida incommunis</i>
<i>Hansenula anomala</i>	20	none	<i>Hyphopichia burtonii</i>	none
<i>Torulopsis maris</i>	21	<i>Candida</i> sp.	<i>Candida maris</i>	<i>Candida maris</i>
<i>Torulopsis glabrata</i>	22	none	<i>Pichia trehalophila</i>	<i>Candida glabrata</i>
<i>Torulopsis candida</i>	23	none	<i>Debaryomyces hansenii</i>	<i>Pichia guilliermondii</i>
<i>Sporobolomyces salmonicolor</i>	24	none	<i>Sporidiobolus johnsonii</i>	<i>Sporidiobolus johnsonii</i>
<i>Saccharomyces cerevisiae</i>	25	<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces cerevisiae</i>
<i>Saccharomyces cerevisiae</i>	26	none	<i>Zygosaccharomyces</i> sp.	<i>Zygosaccharomyces</i> sp.
<i>Rhodotorula minuta</i>	27	none	<i>Rhodotorula aurantiaca</i>	none
<i>Rhodotorula aurantiaca</i>	28	none	<i>Candida</i> sp.	<i>Rhodotorula aurantiaca</i>
<i>Rhodotorula rubra</i>	29	none	none	none
<i>Candida norvegensis</i>	30	none	<i>Candida incommunis</i>	<i>Pichia norvegensis</i>
<i>Candida ciferrii</i>	31	none	<i>Geotrichum terrestre</i>	<i>Stephanosascus ciferrii</i>
<i>Candida utilis</i>	32	none	<i>Pichia jadinii</i>	<i>Pichia jadinii</i>
<i>Candida humicola</i>	33	none	none	<i>Cryptococcus</i> sp.
<i>Cryptococcus albidus</i>	34	<i>Cryptococcus</i> sp.	<i>Cryptococcus albidus</i>	<i>Cryptococcus albidus</i>
<i>Cryptococcus ater</i>	35	none	none	<i>Cryptococcus albidus</i>
<i>Blastoschizomyces capitatus</i>	36	none	<i>Galactomyces geotrichum</i>	<i>Galactomyces geotrichum</i>

Table 1.- Yeast taxa and their identification at 24, 48, and 72 hours.

Name	JSC	24 hr	48 hr	72 hr
<i>Blastoschizomyces capitatus</i>	37	none	none	<i>Galactomyces geotrichum</i>
<i>Blastoschizomyces capitatus</i>	38	<i>Candida pararugosa</i>	<i>Schizoblastosporon starkeyi</i>	<i>Dipodascus ovetensis</i>
<i>Blastoschizomyces capitatus</i>	39	none	<i>Galactomyces geotrichum</i>	<i>Galactomyces geotrichum</i>
<i>Blastoschizomyces capitatus</i>	40	none	<i>Galactomyces geotrichum</i>	<i>Galactomyces geotrichum</i>
<i>Blastoschizomyces capitatus</i>	41	none	none	none
<i>Filobasidium capsuligenum</i>	42	none	<i>Hyphopichia burtonii</i>	<i>Cryptococcus terreus</i>
<i>Pichia farinosa</i>	43	<i>Pichia</i> sp.	none	<i>Pichia farinosa/musicola</i>
<i>Candida intermedia</i>	44	none	none	<i>Pichia mexicana</i>
<i>Torulospora rosei</i>	45	none	none	<i>Schizosaccharomyces pombe</i>
<i>Candida stellatoidea</i>	46	none	none	none
<i>Candida ravautii</i>	47	none	none	none
<i>Candida membranaefaciens</i>	48	none	none	<i>Candida parapsilosis</i>
<i>Candida humicola</i>	50	<i>Trichosporon beigeli</i>	<i>Candida glabrosa</i>	<i>Cryptococcus terreus</i>
<i>Torulopsis candida</i>	51	none	none	<i>Saccharomyces ludwigii</i>
<i>Torulopsis candida</i>	52	none	none	none
<i>Pichia ohmeri</i>	53	none	<i>Candida parapsilosis</i>	<i>Candida parapsilosis</i>
<i>Trichosporon beigeli</i>	54	none	<i>Rhodotorula aurantiaca</i>	none
<i>Candida lusitanae</i>	55	<i>Clavispora lusitanae</i>	<i>Clavispora lusitanae</i>	<i>Clavispora lusitanae</i>

Table 1.- Yeast taxa and their identification at 24, 48, and 72 hours.

Name	JSC	24 hr	48 hr	72 hr
<i>Candida stellatoidea</i>	56	<i>Candida</i> sp.	<i>Hyphopichia burtonii</i>	none
<i>Torulopsis candida</i>	57	<i>Debaryomyces hansenii</i>	<i>Candida salmanticensis</i>	none
<i>Torulopsis candida</i>	58	<i>Candida edax</i>	<i>Debaryomyces hansenii</i>	<i>Trichosporon beigeli</i>
<i>Candida utilis</i>	59	none	none	none
<i>Hyphozyma variabiles</i>	60	<i>Cryptococcus luteolus</i>	<i>Rhodotorula aurantiaca</i>	<i>Pichia mexicana</i>
<i>Hansenula fabianii</i>	61	none	<i>Hyphopichia burtonii</i>	<i>Pichia onychis</i>
<i>Torulospora rosei</i>	62	none	none	<i>Torulaspota globosa</i>
<i>Blastoschizomyces capitatus</i>	63	none	none	none
<i>Candida kefyi</i>	64	<i>Kluyveromyces marxianus</i>	<i>Kluyveromyces marxianus</i>	<i>Kluyveromyces marxianus</i>
<i>Trichosporon beigeli</i>	65	none	<i>Rhodotorula acheniorum</i>	<i>Rhodotorula acheniorum</i>
<i>Cryptococcus ater</i>	66	none	none	none
<i>Torulopsis magnoliae</i>	67	none	<i>Candida magnoliae</i>	<i>Candida magnoliae</i>
<i>Hanseniospora uvarum</i>	68	none	<i>Hanseniaspora guilliermondii/uvarum/valb</i>	<i>Hanseniaspora guilliermondii/uvarum/valb</i>
<i>Candida lusitanae</i>	69	none	<i>Kluyveromyces lodderae</i>	<i>Kluyveromyces lodderae</i>
<i>Candida krusei</i>	70	none	none	none
<i>Candida humicola</i>	71	none	<i>Rhodotorula aurantiaca</i>	<i>Rhodotorula aurantiaca</i>
<i>Trichosporon beigeli</i>	72	<i>Trichosporon beigeli</i>	<i>Rhodotorula acheniorum</i>	none
<i>Candida diddensiae</i>	73	none	<i>Dekkera bruxellensis</i>	none

Table 1.- Yeast taxa and their identification at 24, 48, and 72 hours.

Name	JSC	24 hr	48 hr	72 hr
<i>Metschnikowia pulcherrima</i>	74	none	<i>Candida sorboxylosa</i>	<i>Candida sorboxylosa</i>
<i>Candida albicans</i>	75	<i>Pichia stipitits</i>	<i>Hyphopichia burtonii</i>	<i>Hyphopichia burtonii</i>
<i>Candida albicans</i>	76	none	<i>Hyphopichia burtonii</i>	none
<i>Candida albicans</i>	77	none	<i>Candida albicans</i>	<i>Candida albicans</i>
<i>Candida albicans</i>	78	none	<i>Candida albicans</i>	<i>Candida albicans</i>
<i>Candida albicans</i>	79	<i>Candida sp.</i>	<i>Candida albicans</i>	<i>Candida albicans</i>
<i>Candida lusitaniae</i>	80	none	<i>Candida parapsilosis</i>	<i>Candida parapsilosis</i>
<i>Candida lusitaniae</i>	81	none	<i>Candida parapsilosis</i>	<i>Candida parapsilosis</i>
<i>Candida lusitaniae</i>	82	none	<i>Clavispora lusitaniae</i>	<i>Clavispora lusitaniae</i>
<i>Candida lusitaniae</i>	83	none	<i>Clavispora lusitaniae</i>	<i>Clavispora lusitaniae</i>
<i>Candida krusei</i>	84	none	<i>Issatchenkia orientalis</i>	<i>Issatchenkia orientalis</i>
<i>Candida krusei</i>	85	none	<i>Candida sorboxylosa</i>	<i>Candida sorboxylosa</i>
<i>Candida krusei</i>	86	none	<i>Issatchenkia orientalis</i>	<i>Issatchenkia orientalis</i>
<i>Candida krusei</i>	87	none	<i>Issatchenkia orientalis</i>	<i>Issatchenkia orientalis</i>
<i>Saccharomyces cerevisiae</i>	88	<i>Saccharomyces boulardii</i>	<i>Saccharomyces boulardii</i>	<i>Saccharomyces boulardii</i>
<i>Candida parapsilosis</i>	89	none	<i>Candida parapsilosis</i>	<i>Candida parapsilosis</i>
<i>Candida parapsilosis</i>	90	none	<i>Candida parapsilosis</i>	<i>Candida parapsilosis</i>
<i>Candida parapsilosis</i>	91	none	<i>Candida parapsilosis</i>	<i>Candida parapsilosis</i>

Table 1.- Yeast taxa and their identification at 24, 48, and 72 hours.

Name	JSC	24 hr	48 hr	72 hr
<i>Candida parapsilosis</i>	92	none	<i>Candida parapsilosis</i>	<i>Candida parapsilosis</i>
<i>Candida parapsilosis</i>	93	none	<i>Saccharomyces dairensis</i>	<i>Pichia mexicana</i>
<i>Torulopsis glabrata</i>	94	none	<i>Candida parapsilosis</i>	<i>Candida parapsilosis</i>
<i>Candida tropicalis</i>	95	none	<i>Candida parapsilosis</i>	<i>Candida tropicalis</i>
<i>Cryptococcus neoformans</i>	96	none	<i>Candida albicans</i>	<i>Candida albicans</i>
<i>Cryptococcus neoformans</i>	97	none	none	<i>Filobasidiella neoformans</i> var. <i>neoformans</i>
<i>Blastoschizomyces capitatum</i>	98	none	<i>Schizoblastosporon starkeyi</i>	none
<i>Candida paratropicalis</i>	99	none	<i>Hypopichia burtonii</i>	<i>Cryptococcus terreus</i>
<i>Candida rugosa</i>	100	<i>Candida rugosa</i>	<i>Candida rugosa</i>	<i>Candida rugosa</i>
<i>Torulopsis candida</i>	101	<i>Pichia ohmeri</i>	<i>Pichia ohmeri</i>	<i>Pichia ohmeri</i>
<i>Torulopsis candida</i>	102	none	none	none
<i>Torulopsis candida</i>	103	none	<i>Pichia ohmeri</i>	<i>Pichia ohmeri</i>
<i>Candida krusei</i>	104	none	<i>Issatchenkia orientalis</i>	<i>Issatchenkia</i> sp.
<i>Candida kefyri</i>	105	none	none	<i>Pichia guilliermondii</i>
<i>Candida kefyri</i>	106	none	<i>Guilliermondella selenospora</i>	none
<i>Candida geochares</i>	136	none	<i>Candida geochares</i>	<i>Candida geochares</i>
<i>Yamadazyma haplophilia</i>	138	none	none	<i>Candida parapsilosis</i>
<i>Cryptococcus laurentii</i>	157	<i>Cryptococcus laurentii</i>	<i>Cryptococcus luteolus</i>	<i>Cryptococcus luteolus</i>

Table 1.- Yeast taxa and their identification at 24, 48, and 72 hours.

Name	JSC	24 hr	48 hr	72 hr
<i>Candida albicans</i>	158	none	<i>Candida albicans</i>	<i>Candida albicans</i>